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Brief Research Communication

Systematic Screening for Mutations in the Human Serotonin 1F Receptor Gene in Patients With Bipolar Affective Disorder and Schizophrenia

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Using single strand conformational analysis we screened the complete coding sequence of the serotonin 1F (5-HT_{1F}) receptor gene for the presence of DNA sequence variation in a sample of 137 unrelated individuals including 45 schizophrenic patients, 46 bipolar patients, as well as 46 healthy controls. We detected only three rare sequence variants which are characterized by single base pair substitutions, namely a silent T→A transversion in the third position of codon 261 (encoding isoleucine), a silent C→T transition in the third position of codon 176 (encoding histidine), and a C→T transition in position −78 upstream from the start codon. The lack of significant mutations in patients suffering from schizophrenia and bipolar affective disorder indicates that the 5-HT_{1F} receptor is not commonly involved in the etiology of these diseases.

KEY WORDS: candidate gene, polymorphisms, manic depression, 5-HT_{1F}, single strand conformational analysis

INTRODUCTION

Disturbances of serotonergic pathways have been implicated in many neuropsychiatric disorders, including anxiety, depression, schizophrenia, alcoholism, migraine, aggression, suicidal behavior, and Tourette's syndrome. Molecular biology techniques have revealed that the serotonin (5-hydroxytryptamine, 5-HT) receptors have dramatically more heterogenous subtypes than previously suspected from traditional pharmacologic studies. To date genes coding for five human 5-HT_1 receptor subtypes have been cloned, namely, 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, and 5-HT_{1F} [for review see Shih et al., 1995]. Of these, the genes coding for the 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, and 5-HT_{1E} were previously systematically searched for the presence of genetic variation [for review see Propping and Nöthen, 1995]. So far, only the 5-HT_{1A} and 5-HT_{1E} genes have been screened in bipolar and schizophrenic patients [Erdmann et al., 1995; Shimron-Abarbanell et al., 1995]; however, the results were negative and, thus, mutations in these genes are unlikely to be a common cause for the diseases.

In the present study we sought to identify mutations in the gene coding for the 5-HT_{1F} receptor in bipolar and schizophrenic patients. The human 5-HT_{1F} gene, which has been independently cloned by two laboratories [Adham et al., 1993; Lovenberg et al., 1993], is intronless and encodes a protein 366 amino acids in length.

We investigated genomic DNA samples from 137 probands including patients suffering from bipolar affective disorder (n = 46), schizophrenia (n = 45), as well as healthy control probands (n = 46). The patients were interviewed using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) [Endicott and Spitzer, 1978] and fulfilled DSM-III-R criteria [American Psychiatric Association, 1987]. All individuals were unrelated and of German origin.

Single strand conformational analysis (SSCA) [Orita et al., 1989] was conducted with nine primer pairs designed from the published human 5-HT_{1F} gene sequence [Adham et al., 1993; Lovenberg et al., 1993]. The primers were chosen to produce overlapping fragments encompassing the whole coding region (1101 bp) of the 5-HT_{1F} gene (Table I). Fragment sizes ranged

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TABLE I. PCR Primers for Amplification of Overlapping Fragments Covering the Coding Region of the 5-HT_{1F} Receptor Gene

Primer	Primer sequence	Nucleotide position (5′–3′)	PCR product (bp)
1F-1f	5′-AAAACCTTCAATCTGAACCTCA-3′	−186–−165	249
1F-1r	5′-TGGCATTCTGTTTAACAGTTCC-3′	63–42	
1F-2f	5′-TATATTAATCTTTTAAAACAAAGA-3′	−26–−3	218
1F-2r	5′-GGAACAAATTAAATAATTGG-3′	192–173	
1F-3f	5′-ATCGCTGCAATTATTGTGACC-3′	133–153	254
1F-3r	5′-ACAGCATCTGTGATTGCTCG-3′	386–367	
1F-4f	5′-AAGTGGTCTGTGACATTTGGC-3′	278–298	244
1F-4r	5′-ATGATGCATTCATCATCTCTGC-3′	521–500	
1F-5f	5′-GTATGCCAGGAAAAGGACTCC-3′	390–410	195
1F-5r	5′-GCCAGTGGGATGTAGAAAGC-3′	584–565	
1F-6f	5′-TCATCAAGCACGACCACATT-3′	518–537	197
1F-6r	5′-AGTGCTTTTCTCACCACTCTCC-3′	714–693	
1F-7f	5′-AGGATTGCAAAGGAGGAGGT-3′	655–674	245
1F-7r	5′-CCCAAGATTAATCCCAGGGT-3′	899–880	
1F-8f	5′-AAGATCTCAGGTACAAGAGAACGG-3′	844–867	247
1F-8r	5′-ATCGCACAAGCTTTTGGAAT-3′	1,090–1,071	
1F-9f	5′-AATGTCCAATTTTTTGGCATG-3′	981–1,001	247
1F-9r	5′-AGCCTTATCAATTTAGCAGTTTTC-3′	1,227–1,204	

from 195 to 254 bp. Standard PCR was carried out in a 25 μl total volume, containing 80 ng genomic DNA, 10 pmol of each primer, 1 U Taq DNA polymerase (Life Technologies), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatine, and 0.2 mM dNTPs. Samples were processed in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus). After an initial 5 min denaturation at 94°C, 35 cycles were carried out consisting of 20 sec at 94°C, 20 sec at 57°C (except for fragment 2, 5 and 9, for which annealing temperature was 58°C) and 20 sec at 72°C, followed by a final extension step of 5 min at 72°C.

Four microliters of the PCR product were mixed with 6 μl denaturing solution containing 85% formamide, 1.1% Ficoll 400, 0.001% bromphenolblue, 0.14 × TBE and were boiled 5 min at 95°C. The samples were subsequently chilled on ice and loaded on a 10% polyacrylamide (PAA) gel (acrylamide:bisacrylamide = 49:1; 110 mm × 120 mm × 1.0 mm, Multigel-Long Biometra) containing 0.5 × TBE. Electrophoresis of 16–18 h was performed at 7 V/cm at room temperature and +4°C. The electrophoretic patterns were visualized by silver staining [Budowle et al., 1991]. In total, we found three aberrant SSCA patterns. The first was observed by electrophoresis of fragment 1 at room temperature in two control probands. The second variant appeared as an altered SSCA pattern of fragment 5 at 4°C in a single bipolar patient. The third was recognized by electrophoresis of fragment 7 at 4°C and was found in 2 schizophrenic and 3 bipolar affective patients.

To characterize the underlying DNA sequence variations PCR products from heterozygous individuals were cloned into pUC 18 SmaI/BAP vector (Pharmacia). Single colonies were lysated in 10 μl TE buffer by boiling for 10 min. The lysates were used as template for PCR with primer sets 1F-1f/1F-1r, 1F-5f/1F-5r, and 1F-7f/1F-7r. PCR products with the expected size were analysed by SSCA in order to identify clones containing different alleles. From selected colonies a hemibiotinylated PCR product was generated using one biotinylated vector primer and one normal vector primer. The PCR vector product was incubated with streptavidine Dynabeads (Dynal Ltd.) and magnetic beads were collected with a magnetic concentrator. After washing and denaturing, both strands of DNA were sequenced by the dideoxy nucleotide chain termination method [Sanger et al., 1977] using Sequenase Version 2.0 Kit (U.S. Biochemicals).

The DNA sequence variant in fragment 1 is characterized by a C→T substitution at nucleotide (nt) position −78. The variant found in fragment 5 is a C→T substitution at nt +528 at third position of codon 176 and the variant in fragment 7 is a T→A substitution at nt +783 at the third position of codon 261.

We developed PCR-based restriction fragment length polymorphism assays to confirm the presence of the mutations and to allow rapid genotyping in populations and families. The C→T transition in position −78 creates a recognition site for restriction enzyme HindIII, the C→T transition in position +528 creates a NlaIII restriction site, whereas the T→A transversion in position +783 abolishes an ApoI restriction site. Genomic DNA was amplified with primer sets 1F-1f/1F-1r, 1F-5f/1F-5r, and 1F-7f/1F-7r. Seven microliters of PCR product (fragments 1 and 7) were digested with 3 U of HindIII and 5U ApoI (New England Biolabs), respectively, 2 μl of PCR product (Fragment 5) were digested with 5 U of NlaIII (New England Bio Labs). The digested products were separated on a 10% PAA gel (acrylamide:bisacrylamide 29:1) containing 1 × TBE at 15 V/cm. The restriction profiles were visualized by silver staining [Budowle et al., 1991].

Amplification of genomic DNA using primer set 1F-1f/1F-1r resulted in a 249 bp PCR fragment. Then, depending on the absence or presence of the polymorphic HindIII site, either a fragment of 249 bp (nt −78: C) or two fragments of 144 bp and 105 bp (nt −78: T) are produced. Amplification with primer set 1F-5f/1F-5r re-

sulted in a 195 bp PCR product containing one constant NlaIII restriction site which produces 2 fragments 166 bp and 29 bp in length (nt +528: C). In the presence of the mutation an additional NlaIII restriction site is created which cuts the 166 bp fragment and produces two additional fragments 111 bp and 55 bp in length (nt +528: T). Amplification with primer set 1F-7f/1F-7r resulted in a 245 bp PCR product containing 2 ApoI restriction sites which produce 3 fragments 125 bp, 88 bp, and 32 bp in length (nt +783: T). In the presence of the mutation (nt +783: A) only two fragments 157 bp and 88 bp in length are seen. Digestion patterns of the different genotypes are shown in Figure 1.

The distribution of the sequence variants in the different populations is given in Table II.

In conclusion, we found three rare sequence variants in the human 5-HT$_{1F}$ gene. None of the variants affects the expression or structure of the protein. Our results indicate that the 5-HT$_{1F}$ receptor is not commonly involved in the etiology of schizophrenia and bipolar affective disorder. Our sample size of 45 patients would have allowed a 90% chance of finding a mutation if the frequency of the mutation was at least 5% in the patient sample. However, if the receptor is defective in rare cases, we may have missed such individuals. Furthermore, it cannot be excluded that regulatory sequences such as those in promoter or enhancer sequences may be involved in a subset of patients. Finally, there remains the possibility that we have missed a mutation by relying on SSCA as a mutation screening procedure because the sensitivity is not 100% [Hayashi and Yandell, 1993]. This possibility has been reduced by performing SSCA under two partly different conditions. In fact, all three mutations lead to an altered migration of the PCR fragment only under one of the two conditions applied. However, the existence of undetected variants cannot be completely excluded.

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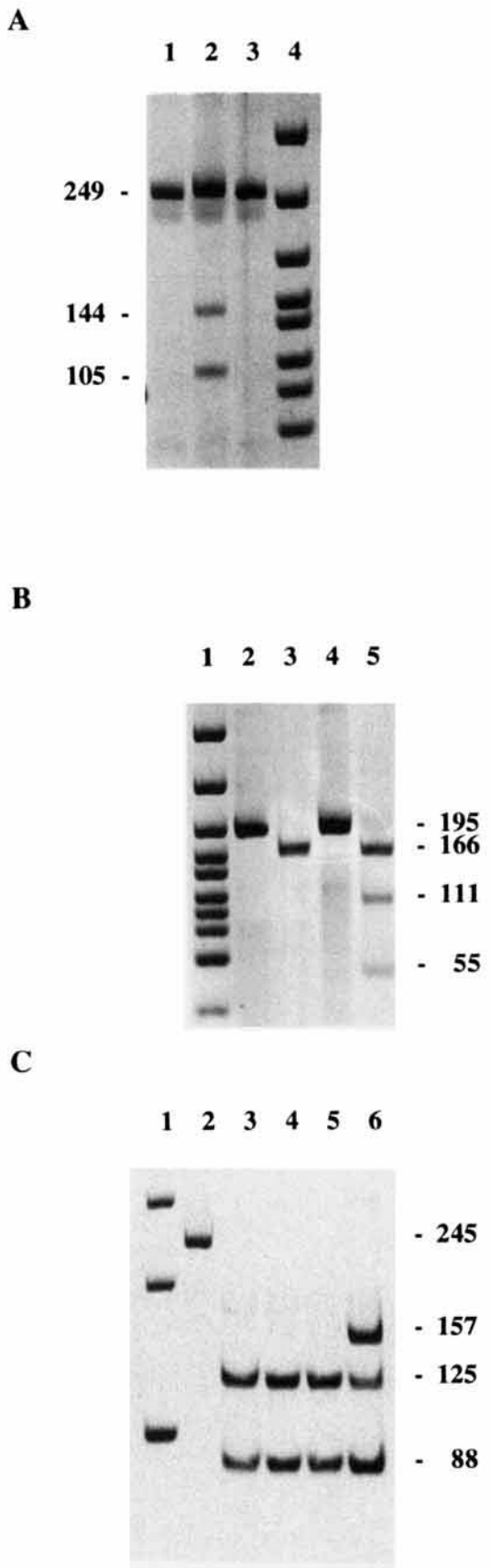

Fig. 1. **A:** HindIII restriction analysis of PCR fragments generated with primer pair 1F-1f/1F-1r to detect the C→T substitution at nt −78. **Lane 1:** Digested PCR product of an individual homozygote for the wild type allele. **Lane 2:** Digested PCR product of an individual heterozygote for the variant allele. **Lane 3:** Undigested PCR product; **lane 4:** ΦX174/HinfI marker (Stratagene). **B:** NlaIII restriction analysis of PCR fragments generated with primer pair 1F-5f/1F-5r to detect the C→T substitution at nt +528. **Lane 1:** ΦX174/HinfI marker (Stratagene). **Lanes 2 and 4:** Undigested PCR products. **Lane 3:** Digested PCR product of an individual homozygote for the wild type allele. **Lane 5:** Digested PCR product of an individual heterozygote for the variant allele. **C:** ApoI restriction analysis of PCR fragments generated with primer pair 1F-7f/1F-7r to reveal the T→A substitution at nt +783. **Lane 1:** 100 bp ladder (Life Technologies). **Lane 2:** Undigested PCR product. **Lanes 3–5:** Digested PCR products of individuals homozygote for the wild type allele. **Lane 6:** Digested PCR product of an individual heterozygote for the variant allele.

TABLE II. Distribution of 5-HT_{1F} Variants in Chromosomes of Patients With Schizophrenia, Bipolar Affective Disorder and Healthy Controls (Allele Frequencies Are Given in Parentheses)

Affection status (no. of alleles)	−78C/T		+528C/T		+783T/A	
	−78C	−78T	+528C	+528T	+783T	+783A
Control (n = 92)	90 (0.98)	2 (0.02)	92 (1.00)	0 (0.00)	92 (1.00)	0 (0.00)
Schizophrenia (n = 90)	90 (1.00)	0 (0.00)	90 (1.00)	0 (0.00)	88 (0.98)	2 (0.02)
Bipolar affective disorder (n = 92)	92 (1.00)	0 (0.00)	91 (0.99)	1 (0.01)	89 (0.97)	3 (0.03)

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